[CANCER RESEARCH 54, 6154–6159, December 1, 1994]

Pan-Fried Meat Containing High Levels of Heterocyclic Aromatic Amines but Low Levels of Polycyclic Aromatic Hydrocarbons Induces Cytochrome P4501A2 Activity in Humans[1]

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ABSTRACT

Heterocyclic aromatic amines (HAAs) are formed when meat juices are pyrolyzed. In humans HAAs are activated *in vivo* by cytochrome P4501A2 (CYP1A2) and *N*-acetyltransferase (NAT2) to mutagens or carcinogens. While activity of NAT2 is noninducible, exposure to cigarettes, polycyclic aromatic hydrocarbons, and cruciferous vegetables has been shown to induce CYP1A2 activity in humans. To date, it is unknown if pan-fried meat, which is consumed at high levels in the United States, is capable of inducing CYP1A2. In order to address this issue, we measured CYP1A2 and NAT2 activities in 66 healthy nonsmokers (33 males and 33 females) in a controlled metabolic feeding study. The study was designed to minimize the influence of known inducers of CYP1A2. Subjects consumed meat pan-fried at a low temperature (100°C) for 7 days followed by 7 days of meat pan-fried at a high temperature (250°C). The low temperature-cooked meat had undetectable levels of HAAs while the high temperature-cooked meat contained high amounts of HAAs [9.0 ng/g of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2.1 ng/g of 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), and 32.8 ng/g of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)]. In contrast, total polycyclic aromatic hydrocarbon content was similar in both meat samples (10.7 ng/g in low temperature-cooked meat and 10.1 ng/g in high temperature-cooked meat). At the end of each period, subjects were tested for CYP1A2 and NAT2 enzyme activity by caffeine metabolism phenotyping. NAT2 activity remained unchanged throughout the study while CYP1A2 activity increased in 47 of 65 (72%) of the subjects after consuming high temperature-cooked meat ($P < 0.0002$), suggesting induction by some compound(s) formed during high temperature cooking. If HAAs are shown to be human carcinogens in epidemiological studies, then meat cooked at high temperatures may pose an increased cancer risk because it contains both inducers of CYP1A2 and procarcinogens MeIQx, DiMeIQx, and PhIP known to be activated by this enzyme.

INTRODUCTION

There are many mutagens and/or carcinogens present in the normal United States diet, some of which are formed during cooking. HAAs[3] are a family of promutagens and procarcinogens formed during the cooking of meats when meat juices pyrolyze (1–5). HAA concentrations in meat increase with longer cooking times and higher temperatures (6, 7). These compounds are among the most potent mutagens tested in the Ames/*Salmonella* bioassay (1) and are carcinogenic in mice, rats, and nonhuman primates (2, 8–11). Furthermore, in some studies weak positive associations between colon cancer and consumption of either "well-done" (12, 13), "browned" (14), "barbecued" (15), or "red meat" (16) have been demonstrated.

The cancer risk to humans posed by HAAs in the diet may depend upon the extent to which the compounds are activated *in vivo* (17). HAAs need to be metabolically activated in order to act as mutagens or carcinogens (18). The initial activation step is thought to be *N*-oxidation by CYP1A2 (19). The *N*-hydroxy arylamine metabolite is *O*-acetylated in the liver or transported to the appropriate target organ where it is *O*-acetylated by the polymorphic NAT2 to form an arylamine-DNA adduct. Both of these enzymes can be measured in the human by evaluating excretion of caffeine metabolites in urine after caffeine consumption (20). The measured phenotype can distinguish between slow and rapid *O*-acetylators and *N*-oxidizers. One study has suggested that people who are rapid metabolizers for both NAT2 and CYP1A2 have increased susceptibility to colon cancer (21).

There is considerable interindividual variability in the activity of both CYP1A2 and NAT2 (20, 22). For NAT2 the variability is due to genetic polymorphisms (23), while for CYP1A2 both genetic and environmental factors are likely to be responsible (20, 24–27). CYP1A2 activity can be affected by factors such as smoking or certain dietary components, *e.g.*, cruciferous vegetables and PAHs (28, 29). However, the ability of pan-fried meat containing high levels of HAAs and minimal levels of PAHs to induce CYP1A2 in humans has not been investigated. In order to address this issue, we designed a human metabolic study to investigate the effect of ingesting meat cooked at high temperature on CYP1A2 and NAT2 activities.

MATERIALS AND METHODS

Study Population. Subjects were recruited from the Beltsville, MD area through advertisements in newsletters at local places of employment, newspapers, and fliers posted at local establishments. Enrollment criteria included: being in good health; being a nonsmoker for at least 6 months; being able to consume caffeine; taking no medication except an occasional analgesic; having weight not less than 90% or greater than 130% of 1983 Metropolitan Life Insurance desirable weights; not consuming any "atypical diet" (including vegetarian); and willing to forego any vitamin or mineral supplements during the study. Five hundred subjects responded to the advertisement and were initially screened via a brief questionnaire for age, height, weight, smoking, and health status. Two hundred thirty-three applicants met the study criteria and were invited to one of several orientation sessions. At the orientation session, subjects received an information package and a detailed description of

Received 6/27/94; accepted 10/3/94.

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[1] This work was performed under interagency agreement between the National Cancer Institute and the United States Department of Agriculture Interagency Agreement Y01-CP2-0521; the National Cancer Institute and the Food and Drug Administration under Interagency Agreement Y01-CP3-0553; and the National Cancer Institute and the United States Department of Energy under Interagency Agreement Y01-CP2-0523-01. This work was also supported by United States Department of Energy under Contract W-7405-Eng-48 and National Cancer Institute Grant CA55861.

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[3] The abbreviations used are: HAAs, heterocyclic aromatic amines; PAHs, polycyclic aromatic hydrocarbons; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; NAT2, *N*-acetyltransferase; CYP1A2, human liver cytochrome P4501A2.

the study objectives, protocol, and requirements of subjects. Subjects who were interested in participating in the study were asked to complete a questionnaire relating to food allergies, medication, general medical history, and availability for the study period.

Ninety-four eligible subjects had a general health assessment, including a medical evaluation by a physician. These subjects also provided blood and urine samples for routine analyses. At this time, the subjects received individual explanations of the study and signed the appropriate consent forms before continuing in the study. Thirty-three men and 33 women were randomly selected from all the subjects with normal medical evaluations and clinical test parameters. The study protocol and consent forms were approved by the Institutional Review Boards of the National Cancer Institute and Georgetown University and by the Human Studies Review Committee of the United States Department of Agriculture. The study protocol also had approval from the Office of Protection of Research Risks, NIH.

Meat Preparation. There were two 7-day controlled dietary periods. The first controlled dietary period contained evening meals with meat cooked at low temperature while the second dietary period contained evening meals with meat cooked at high temperature. For the dietary period using low temperature-cooked meat, the lean (15% fat) ground beef was cooked at 100°C for 20 min to avoid browning and then held in a 90°C oven for 20 min to reduce moisture. For the dietary period using high temperature-cooked meat, the lean ground beef was made into quarter pound patties (0.5 x 4 inches) and pan-fried on a griddle at 250°C for 11 min per side (total of 22 min). The meat patties were finely crumbled in a food processor (Robot Coupe USA, Inc., Jackson, MS). Meats for the two periods were cooked in batches which were divided into 8 equal portions. One portion from each of the batches was mixed for 1 day's diet. Thus, every day in each 7-day dietary period had an equivalent amount of HAAs.

Meat Analysis. Representative samples of low- and high temperature-cooked meat were analyzed for HAAs (6) and PAHs (30) as described previously.

Controlled Dietary Period. Subjects in the metabolic study consumed different amounts of meat depending on their body weight as shown in Table 1. The lightest subject (45.9 kg) consumed 200 g and 180 g of low- and high temperature-cooked meat/day, respectively. The heaviest subject (102.7 kg) consumed 365 g and 328 g of low- and high temperature-cooked meat/day. Therefore, the absolute amount of HAAs varied between the subjects but the dose per unit body weight was relatively constant at 3.6 to 4.4 g meat/kg body weight for low temperature-cooked meat and 3.1 to 4.0 g meat/kg body weight for high temperature-cooked meat. The same amount of meat was consumed every day during the first week and at about a 11% lower amount during the second week. This adjustment in the amount of meat eaten during week 2 was to provide a similar precooked meat weight. Subsequent analysis showed that variation in the absolute amount of meat intake was not significantly associated with the variation in CYP1A2 induction observed from week 1 to week 2 (data not shown).

In order to prevent a lack of variety in food from influencing acceptability of diets, a 7-day menu cycle for the two diet periods was used. The diets were developed for varying caloric intake levels (1600–4400 kcal/day) similar to diets for free-living Beltsville residents. The levels were determined from previous studies conducted at the Beltsville Human Nutrition Research Center and contained approximately 36% kcal from fat, 44% kcal from carbohydrate, 20% kcal from protein, and 7 g of dietary fiber daily. The meals were designed to have the Recommended Dietary Allowances for all the other nutrients. During the two controlled dietary periods, breakfast and dinner were eaten at the Beltsville Human Nutrition Research Center on Monday through Friday. A box lunch was provided at breakfast time. Meals were supervised by registered dietitians. Weekend meals were prepackaged at the facility for home consumption. No food other than that provided by the study was permitted. No alcohol use was permitted during the study. The subjects weighed themselves each day before breakfast. If people were hungry they were moved to a higher calorie level, such that the serving size but not the nature of the food on the menu was changed. However, the amount of meat eaten was kept constant within the week so that the HAA exposure was kept the same for the particular test period.

Table 1 *Amount of meat and heterocyclic aromatic amines consumed daily in the study*

	Low temperature-cooked meat period	High temperature-cooked meat period
Range of meat eaten (g)[a]	200–365	180–328
Range of HAAs eaten (μg)		
MeIQx	0	1.6–3.0
DiMeIQx	0	0.4–0.7
PhIP	0	5.9–10.8

[a] There was a higher amount of meat consumed during the low temperature-cooked meat period compared to high temperature-cooked meat period because of the higher moisture and fat content of the low temperature cooked meat. The amount of meat consumed by subjects was dependent on their body weight. The dose was 3.1–4.4 g of meat/kg of body weight.

Table 2 *Heterocyclic aromatic amine and polycyclic aromatic hydrocarbon content of meat pan-fried at low and high temperature*

	Low temperature-cooked meat[a] (ng/g meat)	High temperature-cooked meat[b] (ng/g met)
HAAs[c]		
MeIQx	ND[d]	9.0
DiMeIQx	ND	2.1
PhIP	ND	32.8
PAHs[e]		
Phenanthrene	5.3	4.9
Fluoranthene	0.3	0.2
Pyrene	2.3	2.7
Benzo(*a*)anthracene	0.9	1.0
Chrysene	0.7	ND
Benzo(*b*)fluoranthene	0.2	0.2
Benzo(*k*)fluoranthene	0.1	0.1
Benzo(*a*)pyrene	0.2	0.2
Benzo(*ghi*)perylene	0.4	0.4
Indenol(123-*cd*)pyrene	0.3	0.4
Total PAHs	10.7	10.1

[a] Ground meat cooked at 100°C for 20 min and reduced moisture in an oven heated to 90°C for 20 min.
[b] Ground meat patties cooked at 250°C for 22 min.
[c] 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-methyl-imidazo[4,5-*f*]quinoline (MeIQ) were not detected in either sample.
[d] ND, Not detected.
[e] Naphthalene, acenaphthene, fluorene, and anthracene were not detected in either sample.

Caffeine Phenotype. The subjects were phenotyped for CYP1A2 and NAT2 function by measuring the ratio of urinary caffeine metabolites at the end of low temperature-cooked meat period (week 1) and at the end of the high temperature-cooked meat period (week 2). At each time point, subjects did not receive any caffeine-containing foods or beverages for 24 h prior to sampling and at least 5 h after taking the test dose. Subjects consumed 3.6 g of instant coffee (114 mg of caffeine) in 266 ml (9 ounces) of water. The subjects voided 4 h after consuming the coffee and then collected their urine over the next h. The urine samples were frozen on dry ice and stored at −70°C for a maximum of 2 weeks. The urine samples were thawed and the pH was adjusted to 3.5 with 1 M HCl and refrozen until analysis. Pooled samples were prepared as a quality control to check for assay reproducibility. Caffeine and its metabolites were extracted and analyzed from the urine as described by Butler *et al.* (20). The phenotype for NAT2 was calculated using the ratio of 5-acetylamino-6-formylamino-3-methyluracil to 1-methylxanthine and the phenotype for CYP1A2 was calculated using the ratio of 1,7-dimethylxanthine plus 1,7-dimethyluric acid to 1,3,7-trimethylxanthine. The coefficient of variation for the quality control samples over the time period of analysis was 8.7% for NAT2 and 9.5% for CYP1A2.

Statistical Analyses. The analysis of the phenotype was performed after a $\log_{10}$ transformation to normalize the data. Tests for a change in phenotype activity were performed using the Wilcoxon signed rank test on the intraindividual differences

$$\Delta = \text{Log}_{10} \text{ week 2 phenotype} - \log_{10} \text{ week 1 phenotype}$$

with 2-sided P values. The Pearson product moment correlation coefficient was used to compare CYP1A2 function at end of week 1 and week 2.

RESULTS

The three main HAAs (Table 2) that were found in the high temperature pan-fried cooked meat were MeIQx, DiMeIQx, and PhIP.

The elevated levels of HAAs found in the high temperature-cooked meats are in the range consumed by people who eat very well done grilled meat or very crisp bacon.[4] There was no detectable MeIQx, DiMeIQx, or PhIP in the low temperature-cooked meat.

Fourteen PAHs (Table 2) were measured; total PAH concentrations in the low and high temperature-cooked meat samples were similar (10.7 and 10.1 ng/g, respectively). Benzo(*a*)pyrene levels, which are used as a general measure of five-ring PAH compounds, were identical in the two samples (0.2 ng/g). Total PAH levels in the non-meat part of the diet were also measured. The concentrations were 9.1 ng/g during the first week as compared to 9.0 ng/g during the second week.

The mean, standard error, median, and range for NAT2 and CYP1A2 for the low temperature-cooked meat period (week 1) and high temperature-cooked meat period (week 2) are given in Table 3. NAT2 activity did not change appreciably between week 1 and week 2 measurements ($P = 0.45$). In contrast, there was a 50% increase in CYP1A2 activity from week 1 to week 2. No gender-specific induction was observed in these subjects. Mean CYP1A2 activities for males and females at the end of week 1 were 9.96 and 8.63, respectively. At the end of week 2 the mean CYP1A2 activity was 13.42 for males and 12.71 for females.

Fig. 1 illustrates the change in the overall distribution of CYP1A2 in the study population, which was shifted to a higher value after subjects consumed high temperature-cooked meat for 7 days. Fig. 2

Table 3 *Summary statistics of acetyltransferase and cytochrome P4501A2 activities measured by urinary excretion of caffeine metabolites*

	End of low temperature-cooked meat period (Day 7)	End of high temperature-cooked meat period (Day 14)
	Molar ratio[a] Mean ± SE Median (range)	Molar ratio[a] Mean ± SE Median (range)
NAT2[b]	1.12 ± 0.120 0.60 (0.19–3.70)	1.10 ± 0.118 0.46 (0.16–3.80)
CYP1A2[c]	9.30 ± 0.598 8.85 (2.10–28.0)	13.06 ± 0.723 13.00 (2.50–34.0)

[a] The phenotype for NAT2 was calculated using the molar ratio of 5-acetylamino-6-formylamino-3-methyluracil to 1-methylxanthine; the phenotype for CYP1A2 was calculated using the molar ratio of 1,7-dimethylxanthine plus 1,7-dimethyluric acid to 1,3,7-trimethylxanthine.

[b] Difference between the two periods was not statistically significant ($P = 0.45$) by Wilcoxon signed rank test, $n = 65$.

[c] Difference between the two periods was statistically significant ($P < 0.0002$) by Wilcoxon signed rank test, $n = 65$.

presents the absolute change (log week 2 activity − log week 1 activity) in CYP1A2 function for each of the 65 individuals with complete data. The CYP1A2 function increased in 47 subjects (72%) and decreased in 18 subjects (28%) at week 2 compared to week 1 ($P < 0.0002$ by Wilcoxon signed rank test).

Fig. 3 presents the percentage change

$$\frac{\text{Week 2} - \text{Week 1}}{\text{Week 1}} \times 100$$

in CYP1A2. Of the 47 subjects who had increased CYP1A2 function, 36 subjects (55%) increased over 30% of their week 1 CYP1A2 function. In contrast, only 2 subjects (3%) decreased over 30%.

The intraindividual correlation coefficient of NAT2 phenotype measured in week 1 and that measured in week 2 was 0.96 ($P < 0.0001$). The correlation of CYP1A2 phenotype measured during the same time periods was substantially lower at 0.54 but still highly significant ($P < 0.0001$). Fig. 4, a graph of CYP1A2 function in week 1 *versus* week 2, demonstrates this intraindividual effect: people low in CYP1A2 function in week 1 tended to remain low in week 2; and people high in CYP1A2 function in week 1 tended to be high in week 2.

DISCUSSION

This is the first report in humans of CYP1A2 induction caused by high temperature-cooked meat containing minimal PAH levels. In

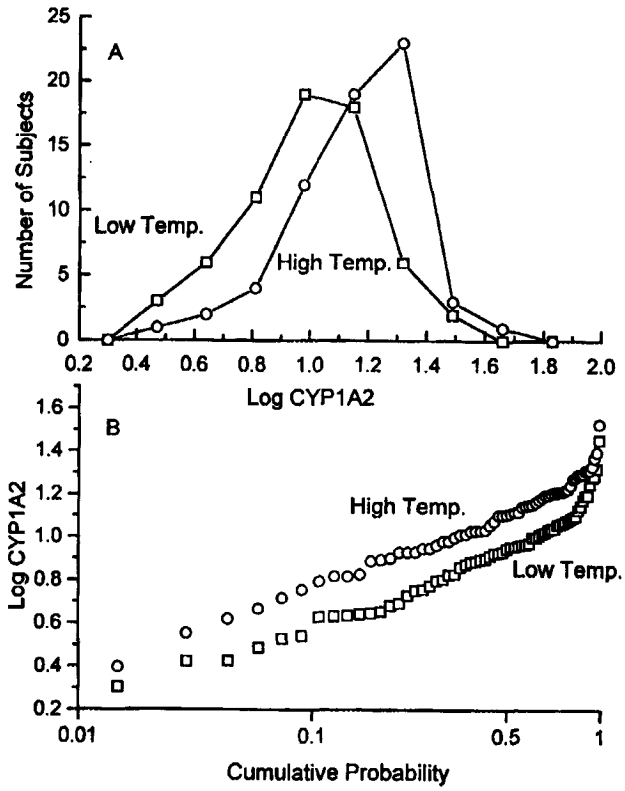

Fig. 1. *A*, frequency distribution of log CYP1A2 activity for 66 subjects after 7 days of consuming low temperature-cooked meat (week 1) and 7 days of consuming high temperature-cooked meat (week 2). CYP1A2 activity defined as the molar ratio of 1,7-dimethylxanthine plus 1,7-dimethyluric acid to 1,3,7-trimethylxanthine. *B*, probit analyses of log CYP1A2 activity at end of weeks 1 and 2.

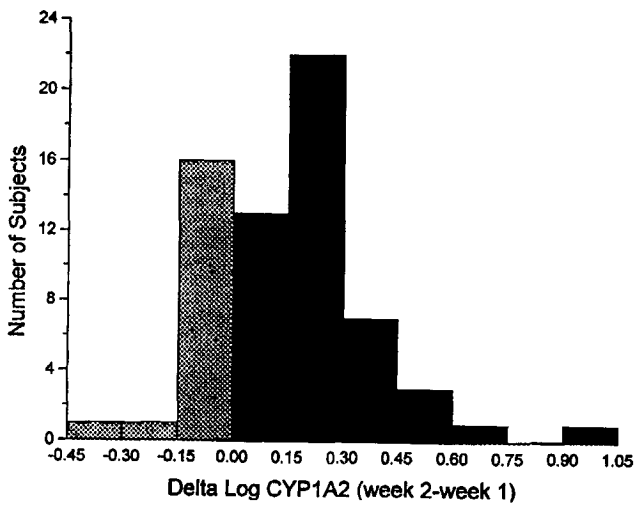

Fig. 2. Frequency distribution of delta (log CYP1A2 at the end of week 2 − log CYP1A2 at the end of week 1) for each individual. $P < 0.0002$ for Wilcoxon signed rank test. ▨, Δ below 0; ■, Δ above 0.

[4] Unpublished data.

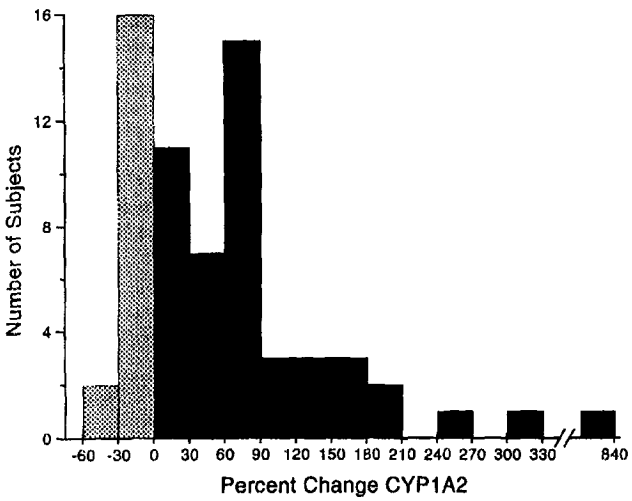

Fig. 3. Percentage change in CYP1A2 activity

$$\frac{\text{Week 2 CYP1A2} - \text{Week 1 CYP1A2}}{\text{Week 1 CYP1A2}} \times 100$$

▒, percentage change below 0; ■, percentage change above 0.

1976, Conney *et al.* (31) reported that meat cooked directly over burning charcoal was capable of inducing CYP1A2 in humans (as measured by phenacetin metabolism) after daily consumption for 4 days. They concluded that PAHs probably contributed to this induction effect, since these compounds are present at high levels in barbecued meat and consistently induce P4501A activity in rodent and *in vitro* models (28). In the current study, we minimized and prevented changes in PAH exposure. PAH levels in food samples consumed during the study were quite low compared to levels generally found in barbecued meat [0.2 ng/g in the current study *versus* 2–4 ng/g in barbecued samples analyzed by the same laboratory (32, 33)] and did not change appreciably from week 1 to week 2. As such, the induction effect in CYP1A2 that we observed during the study period was probably not due to changes in dietary PAH exposure. Furthermore, CYP1A2 activity has been shown to be affected by various other environmental factors (22). This metabolic study was designed to minimize the influence of other known inducers of CYP1A2, such as smoking (34, 35), cruciferous vegetables (28), and possible modifiers such as alcohol, vitamin and mineral supplements, and oral contraceptives or postmenopausal estrogens.

Animal studies provide evidence that HAAs induce CYP1A2 (36–43), although it is likely that other unknown compound(s) formed during high temperature cooking also serve as inducers of CYP1A2. From a public health perspective, however, it does not matter which compound causes the induction effect, given that humans consume complex mixtures of compounds in their diet and not pure HAAs. The main finding of this study is that meat samples cooked at a high temperature contain some component that induces CYP1A2, which is of particular concern given that the same meat samples also contain procarcinogens (MeIQx, DiMeIQx, and PhIP) activated by CYP1A2.

In contrast to CYP1A2, NAT2 activity did not change throughout the study. This finding is consistent with extensive evidence that interindividual differences in NAT2 are due to autosomal inheritance of a phenotype which cannot be altered by environmental factors (22, 23).

Since in this study logistic considerations required giving all subjects the low temperature-cooked meat first and the high temperature-cooked meat second, we cannot exclude the possibility that the intra-individual changes in CYP1A2 were due to a period effect and not to the change in the diet. However, we conclude that diet was the cause of the increase since no other factors could be identified that changed during the 2-week study period which might have influenced this enzyme activity. Furthermore, no consistent change was observed in a study where CYP1A2 function was measured multiple times in the same individuals over a similar time frame (20, 27).

The demonstration of inducibility suggests that recent dietary exposure can affect CYP1A2 function. However, the positive correlation ($r = 0.54$) of CYP1A2 function within individuals suggests that the variability we found in free-living individuals was not entirely due to such environmental factors. This "fixed" component may be mediated by genetic, environmental, or dietary factors which need longer than 7 days to influence the CYP1A2 function. Family and twin studies have provided suggestive but as yet inconclusive evidence for genetic (hereditary) control of CYP1A2 activity (27, 44). At present, efforts are under way in several laboratories to understand the genetic basis of the fixed component of the phenotype for CYP1A2. This polymorphism may involve the CYP1A2 structural gene (27), its regulatory component, or its relevant receptor.

Lastly, interindividual CYP1A2 activity varied 15-fold among the 66 subjects involved in the study. This compares to an observed 40–60-fold range in hepatic CYP1A2 levels, based either on enzyme activity or on immunoreactive protein content (45–49). Moreover, for those 36 individuals showing appreciable CYP1A2 induction, the percentage change ranged from 30 to 840% (median, 75%). These data are comparable to those reported for CYP1A2 induction by cigarette smoke, where the increase in the mean CYP1A2 activity was also 75% (21). In the liver, this is known to correspond to a 3.5–4.2-fold induction of CYP1A2 activity or protein content (47). The resultant increase in carcinogen activation (*i.e.*, arylamine *N*-oxidation) has been studied further in a pharmacokinetic model and was shown to result in similar increases in carcinogen-DNA adduct formation (50). Although the biological consequences of this variation are not fully understood, Lang *et al.* (21) have reported that high CYP1A2 (and NAT2) activity is associated with increased colorectal cancer risk, especially when combined with a dietary preference of

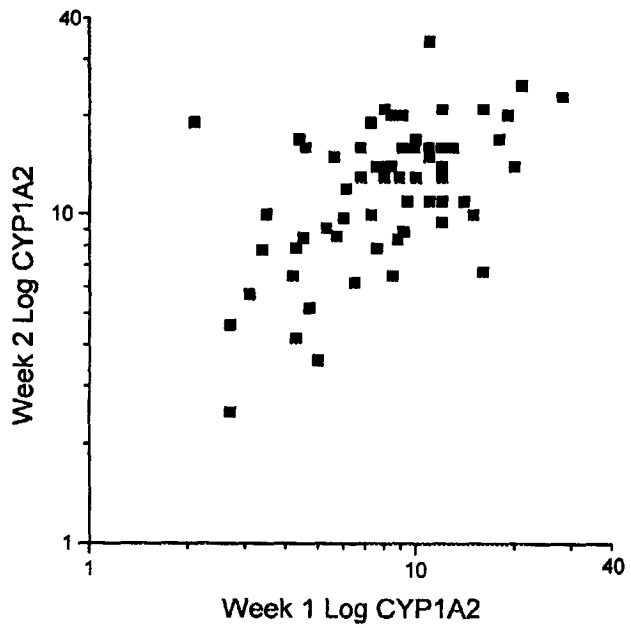

Fig. 4. Correlation between CYP1A2 activity at the end of week 1 and week 2. Within person correlation, $r = 0.54$, $P < 0.001$.

well-done red meat. We are currently following up these findings to further explore the interaction of CYP1A2 (and NAT2) activity with dietary consumption of HAAs for cancer risk in humans.

In conclusion, we observed an increase in CYP1A2 activity in the majority of people who consumed high temperature-cooked meat that contained large amounts of HAAs. If HAAs are human carcinogens, meat cooked at high temperatures may pose an increased cancer risk, because it contains both inducers of CYP1A2 and procarcinogens (MeIQx, DiMeIQx, and PhIP) known to be activated by this enzyme. Studies are also in progress to evaluate the presence of HAAs in meat samples cooked by a variety of techniques in order to understand how widespread these compounds are in the general diet.

ACKNOWLEDGMENTS

We are grateful to the subjects for participating in the study and to P. Steele and the staff at Human Study Facility, United States Department of Agriculture, Beltsville, MD, for the efficient implementation of the dietary component. We thank D. G. Rhodes, V. C. Morris, M. Morris, and A. D. Hill for technical assistance and A. Greenberg and C-H. Hsu of Rutgers University for polycyclic aromatic hydrocarbon analysis.

REFERENCES

1. Sugimura, T., Sato, S., and Wakabayashi, K. Mutagens/carcinogens in pyrolysate of amino acids and proteins and in cooked foods: heterocyclic aromatic amines. *In:* Y. T. Woo, D. Y. Lai, J. C. Arcos, and M. F. Argue (eds.), Chemical Induction of Cancer, Structural Bases and Biological Mechanisms, pp. 681–710. New York: Academic Press, Inc., 1988.
2. Adamson, R. H. Mutagens and carcinogens formed during cooking of food and methods to minimize their formation. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Cancer Prevention, pp. 1–7. Philadelphia: J. B. Lippincott Co., 1990.
3. Felton, J. S., and Knize, M. G. Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. Mutat. Res., *259:* 205–217, 1991.
4. Felton, J. S., and Knize, M. G. New mutagens from cooked food. Prog. Clin. Biol. Res., *347:* 19–38, 1990.
5. Hatch, F. T., Knize, M. G., Moore, D. H., and Felton, J. S. Quantitative correlation of mutagenic and carcinogenic potencies for heterocyclic amines from cooked foods and additional aromatic amines. Mutat. Res., *271:* 269–287, 1992.
6. Gross, G. A., and Gruter, A. Quantitation of mutagenic/carcinogenic heterocyclic amines in food products. J. Chromatogr., *592:* 271–278, 1992.
7. Knize, M. G., Dolbeare, F. A., Carroll, K. L., and Felton, J. S. Effects of cooking time and temperature on the heterocyclic amine content of fried-beef patties. Food Chem. Toxicol., *32:* 595–603, 1994.
8. Adamson, R. H., Snyderwine, E. G., Thorgeirsson, U. P., Schut, H. A. J., Turesky, R. J., Thorgeirsson, S. S., Takayama, S., and Sugimura, T. Metabolic processing and carcinogenicity of heterocyclic amines in non-human primates. *In:* L. Ernster, H. Esumi, and Y. Fujii (eds.), Xenobiotics and Cancer, pp. 289–301. Tokyo: Japan Scientific Societies Press, 1991.
9. Ohgaki, H., Hasegawa, H., Kato, T., Suenaga, M., Ubukata, M., Sato, S., Takayama, S., and Sugimura, T. Carcinogenicity in mice and rats of heterocyclic amines in cooked foods. Environ. Health Perspect., *67:* 129–134, 1986.
10. Ohgaki, H., Takayama, S., and Sugimura, T. Carcinogenicities of heterocyclic amines in cooked food. Mutat. Res., *259:* 399–410, 1991.
11. Ito, N., Hasegawa, R., Sano, M., Tamano, S., Esumi, H., Takayama, S., and Sugimura, T. A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Carcinogenesis (Lond.), *12:* 1503–1506, 1991.
12. Schiffman, M. H., Van Tassell, R., and Andrew, A. W. Epidemiologic studies of fecal mutagenicity, cooked meat ingestion, and risk of colorectal cancer. Mutat. Environ., 205–214, 1990.
13. Schiffman, M. H., and Felton, J. S. Fried foods and the risk of colon cancer. Am. J. Epidemiol., *131:* 376–378, 1990.
14. Gerhardsson De Verdier, M., Hagman, U., Peters, R. K., Steineck, G., and Overvik, E. Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm. Int. J. Cancer, *49:* 520–525, 1991.
15. Peters, R. K., Garabrant, D. H., Yu, M. C., and Mack, T. M. A case-control study of occupational and dietary factors in colorectal cancers in young men by subsite. Cancer Res., *49:* 5459–5468, 1989.
16. Giovannucci, E., Rimm, E. B., Stamfer, M. J., Colditz, G. A., Ascherio, A., and Willet, W. C. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res., *54:* 2390–2397, 1994.
17. Degawa, M., Tanimura, S., Agatsuma, T., and Hashimoto, Y. Hepatocarcinogenic heterocyclic aromatic amines that induce cytochrome P-448 isozymes, mainly cytochrome P-448H (P-4501A2), responsible for mutagenic activation of the carcinogenesis in rat liver. Carcinogenesis (Lond.), *10:* 1119–1122, 1989.
18. Kato, R. Metabolic activation of mutagenic heterocyclic amines from protein pyrolysates. CRC Crit. Rev. Toxicol., *16:* 307–348, 1986.
19. Turesky, R. J., Lang, N. P., Butler, M. A., Teitel, C. H., and Kadlubar, F. F. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. Carcinogenesis (Lond.), *12:* 1839–1845, 1991.
20. Butler, M. A., Lang, N. P., Young, J. F., Caporaso, N. E., Vineis, P., Hayes, R. B., Teitel, C. H., Massengill, J. P., Lawsen, M. F., and Kadlubar, F. F. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. Pharmacogenetics, *2:* 116–127, 1992.
21. Lang, N. P., Butler, M. A., Massengill, J., Lawson, M., Stotts, R. C., Hauser-Jensen, M., and Kadlubar, F. F. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increases the risk for colorectal cancer or polyps. Cancer Epidemiol., Biomarkers & Prev., *3:* 675–682, 1994.
22. Daly, A. K., Cholerton, S., Gregory, W., and Idle, J. R. Metabolic polymorphisms. Pharmacol. Ther., *57:* 129–160, 1993.
23. Caporaso, N., Landi, M. T., and Vineis, P. Relevance of metabolic polymorphisms to human carcinogenesis: evaluation of epidemiologic evidence. Pharmacogenetics, *1:* 4–19, 1991.
24. Guengerich, F. P. Cytochrome P450 enzymes. Am. Sci., *81:* 440–447, 1993.
25. Guengerich, F. P. Human cytochrome P-450 enzymes. Life Sci., *50:* 1471–1478, 1992.
26. Gonzalez, F. J. Human cytochromes P450: problems and prospects. Trends Pharmacol. Sci., *13:* 346–351, 1992.
27. Nakajima, M., Yokoi, T., Mizutani, M., Shin, S., Kadlubar, F. F., and Kamataki, T. Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the CYP1A2 gene. Cancer Epidemiol., Biomarkers & Prev., *3:* 413–421, 1994.
28. Conney, A. H. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. Cancer Res., *42:* 4875–4917, 1982.
29. Welch, R. M., Gommi, B., Alvares, A. P., and Conney, A. H. Effect of enzyme induction on the metabolism of benzo(*a*)pyrene and 3′-methyl-4-monomethylaminoazobenzene in the pregnant and fetal rat. Cancer Res., *32:* 973–978, 1972.
30. Greenberg, A., Hsu, C-H., Rothman, N., and Strickland, P. T. PAH profiles of charbroiled hamburgers Pyrene/B[A]P ratios and presence of reactive PAH. Polycyclic Aromatic Compounds, *3:* 101–110, 1993.
31. Conney, A. H., Pantuck, E. J., Hsiao, K. C., Garland, W. A., Anderson, K. E., Alvares, A. P., and Kappas, A. Enhanced phenacetin metabolism in human subjects fed charcoal-broiled beef. Clin. Pharmacol. Ther., *20:* 633–642, 1976.
32. Rothman, N., Poirier, M., Baser, M., Hansen, J., Gentile, C., Bowman, E. D., and Strickland, P. T. Formation of polycyclic aromatic hydrocarbons-DNA adducts in peripheral white blood cells during consumption of charcoal-broiled beef. Carcinogenesis (Lond.), *11:* 1241–1243, 1990.
33. Buckley, T. J., and Lioy, P. J. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Br. J. Ind. Med., *49:* 113–124, 1992.
34. Welch, R. M., Harrison, Y. E., Gommi, B. W., Poppers, P. J., Finster, M., and Conney, A. H. Stimulatory effect of cigarette smoking on the hydroxylation of 3,4-benzpyrene and *N*-demethylation of 3-methyl-4-monomethylaminoazobezene by enzymes in human placenta. Clin. Pharmacol. Ther., *10:* 100–109, 1969.
35. Welch, R. M., and Conney, A. H. Cigarette smoke: stimulatory effect on metabolism of 3,4-benzpyrene by enzymes in rat lung. Life Sci., *10:* 215–221, 1971.
36. Degawa, M., Tanimura, S., Agatsuma, T., and Hashimoto, Y. Hepatocarcinogenic heterocyclic aromatic amines that induce cytochrome P-488 isoenzymes, mainly cytochrome P-448H (P-450IA2), responsible for mutagenic activation of the carcinogens in rat liver. Carcinogenesis (Lond.), *10:* 1119–1122, 1989.
37. Degawa, M., Kobayashi, K., Miura, S., Arai, H., Esumi, H., Sugimura, T., and Hashimoto, Y. Species difference among experimental rodents in induction of P450IA family enzymes by 2-amino-1-methyl-6-phenylinidazo[4,5-*b*]pyridine. Jpn. J. Cancer Res., *83:* 1047–1051, 1992.
38. Rodrigues, A. D., Ayrton, A. D., Williams, E. J., Lewis, D. F. V., Walker, R., and Ioannides, C. Preferential induction of rat hepatic P450 I proteins by food carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline. Eur. J. Biochem., *181:* 627–631, 1989.
39. Kleman, M. I., Overvik, E., Mason, G. F., and Gustafsson, J. *In vitro* activation of the dioxin receptor to a DNA-binding form by food-borne heterocyclic amines. Carcinogenesis (Lond.), *13:* 1619–1624, 1992.
40. Kleman, M., Overvik, E., Mason, G., and Gustafsson, J. Effects of the food mutagens MeIQx and PhIP on the expression of cytochrome P450IA proteins in various tissues of male and female rats. Carcinogenesis (Lond.), *11:* 2185–2189, 1990.
41. Adachi, H., Degawa, M., Miura, S., Hashimoto, Y., Sugimura, T., and Esumi, H. Induction of putative new cytochrome P450 isoenzyme in rat liver by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. Biochem. Biophys. Res. Commun., *174:* 797–803, 1991.
42. Degawa, M., Hishinuma, T., Yoshida, H., and Hashimoto, Y. Species, sex and organ differences in induction of a cytochrome P-450 isoenzyme responsible for carcinogen activation: effect of dietary hepatocarcinogenic tryptophan pyrolysate components in mice and rats. Carcinogenesis (Lond.), *8:* 1913–1918, 1987.
43. Sinclair, J. F., Schaeffer, B. K., Wood, S. G., Lambrecht, L. K., Gorman, N., Bement, W. J., Smith, E. L., Sinclair, P. R., and Waldern, C. A. 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline induces and inhibits cytochrome P450 from the IA subfamily in chick and rat hepatocytes. Cancer Res., *52:* 3615–3621, 1992.
44. Penno, M. B., Dvorchik, B. H., and Vesell, E. S. Genetic variation in rates of antipyrine metabolite formation: a study in uninduced twins. Proc. Natl. Acad. Sci. USA, *78:* 5193–5196, 1981.
45. Hammons, G. J., Guengerich, F. P., Weis, C. C., Beland, F. A., and Kadlubar, F. F. Metabolic oxidation of carcinogenic arylamines by rat, dog, and human hepatic microsomes and by purified flavin-containing and cytochrome P-450 monooxygenases. Cancer Res., *45:* 3578–3585, 1985.

46. Minchin, R. F., McManus, M. E., Boobis, A. R., Davies, D. S., and Thorgeirsson, S. S. Polymorphic metabolism of the carcinogen 2-acetylaminofluorene in human liver microsomes. Carcinogenesis (Lond.), *6:* 1721–1724, 1985.
47. Sesardic, D., Boobis, A. R., Edwards, R. J., and Davies, D. S. A form of cytochrome P450 in man, orthologous to form d in the rat, catalyzes the *O*-deethylation of phenacetin and is inducible by cigarette smoking. Br. J. Clin. Pharmacol., *26:* 363–372, 1988.
48. Butler, M. A., Guengerich, F. P., and Kadlubar, F. F. Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4′-methylenebis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochromeP-450 monooxygenases. Cancer Res., *49:* 25–31, 1989.
49. Butler, M. A., Iwasaki, M., Guengerich, F. P., and Kadlubar, F. F. Human cytochrome P-450$_{PA}$ (P-450IA2), the phenacetin *O*-deethylase, is primarily responsible for hepatic 3-demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. Proc. Natl. Acad. Sci. USA, *86:* 7696–7700, 1989.
50. Kadlubar, F. F., Dooley, K. L., Teitel, C. H., Roberts, D. W., Benson, R. W., Butler, M. A., Bailey, J. R., Young, J. F., Skipper, P. W., and Tannenbaum, S. R. Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladder DNA adduct level in beagle dogs given the carcinogen 4-aminobiphenyl. Cancer Res., *51:* 4371–4377, 1991.